

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

Claim 1 (currently amended): A Pol I type DNA polymerase nucleic acid ~~polymerase~~ comprising a modification that increases or enhances fidelity, wherein said modification corresponds to

amino acid position Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Asn, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr, and Val, or

amino acid position Lys726 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val, or

amino acid positions Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val and Lys726 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val, or

amino acid positions Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val and Phe730 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the

group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, and Val

~~of a *Thermotoga neapolitana* polymerase, with the proviso that when said modification consists of a single amino acid substitution at either Arg722 or Lys726, said substitution is an amino acid other than Ala, and with the proviso that when said modification consists of an amino acid substitution at Arg722, said substitution is an amino acid other than Pro, Trp, and Gln.~~

Claim 2 (currently amended): A Pol I type DNA polymerase nucleic acid polymerase comprising a modification that reduces or eliminates misincorporation of nucleotides during nucleic acid synthesis, wherein said modification corresponds to amino acid position Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Asn, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr, and Val, or

amino acid position Lys726 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val, or

amino acid positions Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val and Lys726 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val, or

amino acid positions Arg722 of a *Thermotoga neapolitana* polymerase
substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys,
Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val and Phe730 of a
Thermotoga neapolitana polymerase substituted with an amino acid selected from the
group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Pro,
Ser, Thr, Trp, Tyr, and Val

~~of a *Thermotoga neapolitana* polymerase, with the proviso that when said~~
~~modification consists of a single amino acid substitution at either Arg722 or Lys726, said~~
~~substitution is an amino acid other than Ala, and with the proviso that when said~~
~~modification consists of an amino acid substitution at Arg722, said substitution is an~~
~~amino acid other than Pro, Trp, and Gln.~~

Claim 3 (cancelled)

Claim 4 (currently amended): The polymerase of claim 1 or 2 [[3]], wherein said
polymerase is derived from a mesophilic organism or said polymerase is thermostable.

Claim 5 (currently amended): The polymerase of claim 1 or claim 2 [[3]],
wherein said polymerase is derived from a polymerase selected from the group
consisting of Tne DNA polymerase, Taq DNA polymerase, Tma DNA polymrase, Tth
DNA polymerase, Tli (VENTTM) DNA polymerase, Pfu DNA polymerase,
DEEPVENTTM DNA polymerase, Pwo DNA polymerase, Bst DNA polymerase, Bca
DNA polymerase, Tfl DNA polymerase, ~~and mutants and fragments thereof.~~

Claim 6 (previously presented): The polymerase of claim 1 or 2, further comprising one or more modifications to reduce or eliminate one or more activities selected from the group consisting of:

- (a) the 3'→5' exonuclease activity of the polymerase;
- (b) the 5'→3' exonuclease activity of the polymerase; and
- (c) the discriminatory activity against one or more dideoxynucleotides.

Claim 7 (previously presented): The polymerase of claim 1 or claim 2, wherein said polymerase is modified to reduce or eliminate 3'→5' exonuclease activity.

Claim 8 (previously presented): The polymerase of claim 1 or claim 2, wherein said polymerase is modified to reduce or eliminate discriminatory activity against one or more dideoxynucleotides.

Claim 9 (previously presented): The polymerase of claim 1 or claim 2, wherein said polymerase is modified to reduce or eliminate 5'→3' exonuclease activity.

Claims 10-13 (cancelled)

Claim 14 (currently amended): The polymerase of claim 1 or claim 2 [[12]], wherein Arg722 is substituted with an amino acid selected from the group consisting of Ala, -Asn, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr and Val, ~~with the proviso that when said modification consists of a single amino acid substitution, said substitution is not Ala.~~

Claims 15-16 (cancelled)

Claim 17 (currently amended): The polymerase of claim 1 or claim 2 [[15]], wherein Lys726 is substituted with an amino acid selected from the group consisting of ~~Ala~~, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, ~~with the proviso that when said modification consists of a single amino acid substitution, said substitution is not Ala.~~

Claims 18-19 (cancelled)

Claim 20 (currently amended): The polymerase of claim 1 or claim 2 [[18]], wherein Arg722 is substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, and wherein Lys726 is substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val.

Claims 21-36 (cancelled)

Claim 37 (previously presented): A kit for amplifying, synthesizing, or sequencing a DNA molecule comprising one or more of the modified polymerases of claim 1 or claim 2.

Claim 38 (original): The kit of claim 37, further comprising one or more dideoxyribonucleoside triphosphates.

Claim 39 (original): The kit of claim 37, further comprising one or more deoxyribonucleoside triphosphates.

Claim 40 (original): The kit of claim 38, further comprising one or more deoxyribonucleoside triphosphates.

Claims 41-46 (cancelled)

Claim 47 (currently amended): A thermostable Pol I type DNA polymerase ~~nucleic acid polymerase~~ comprising a modification that corresponds to

amino acid position Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Asn, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr, and Val, or

amino acid position Lys726 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val, or

amino acid positions Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val and Lys726 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the

group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val, or

amino acid positions Arg722 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val and Phe730 of a *Thermotoga neapolitana* polymerase substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, and Val

~~of a *Thermotoga neapolitana* polymerase, with the proviso that when said modification consists of a single amino acid substitution at either Arg722 or Lys726, said substitution is an amino acid other than Ala, and with the proviso that when said modification consists of an amino acid substitution at Arg722, said substitution is an amino acid other than Pro, Trp, and Gln.~~

Claims 48-49 (cancelled)

Claim 50 (currently amended): The polymerase of claim 47 [[48]], wherein said polymerase is derived from a polymerase selected from the group consisting of Tne DNA polymerase, Taq DNA polymerase, Tma DNA polymerase, Tth DNA polymerase, Tli (VENTTM) DNA polymerase, Pfu DNA polymerase, DEEPVENTTM DNA polymerase, Pwo DNA polymerase, Bst DNA polymerase, Bca DNA polymerase, Tfl DNA polymerase, ~~and mutants and fragments thereof.~~

Claim 51 (previously presented): The polymerase of claim 47, further comprising one or more modifications to reduce or eliminate one or more activities selected from the group consisting of:

- (a) the 3'→5' exonuclease activity of the polymerase;
- (b) the 5'→3' exonuclease activity of the polymerase; and
- (c) the discriminatory activity against one or more dideoxynucleotides.

Claim 52 (previously presented): The polymerase of claim 47, wherein said polymerase is modified to reduce or eliminate 3'→5' exonuclease activity.

Claim 53 (previously presented): The polymerase of claim 47, wherein said polymerase is modified to reduce or eliminate discriminatory activity against one or more dideoxynucleotides.

Claim 54 (previously presented): The polymerase of claim 47, wherein said polymerase is modified to reduce or eliminate 5'→3' exonuclease activity.

Claims 55-56 (cancelled)

Claim 57 (currently amended): The polymerase of claim 47 ~~[[56]]~~, wherein Arg722 is substituted with an amino acid selected from the group consisting of ~~Ala~~, Asn, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr and Val, ~~with the proviso that when said modification consists of a single amino acid substitution, said substitution is not Ala.~~

Claim 58 (cancelled)

Claim 59 (currently amended): The polymerase of claim 47 [[58]], wherein Lys726 is substituted with an amino acid selected from the group consisting of ~~Ala~~, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, ~~with the proviso that when said modification consists of a single amino acid substitution, said substitution is not Ala.~~

Claim 60 (cancelled)

Claim 61 (currently amended): The polymerase of claim 47 [[60]], wherein Arg722 is substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, and wherein Lys726 is substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val.

Claims 62-63 (cancelled)

Claim 64 (previously presented): The polymerase of claim 47, wherein said polymerase has substantially reduced or lacks 3'→5' exonuclease activity.

Claim 65 (previously presented): A kit for amplifying, synthesizing, or sequencing a DNA molecule comprising one or more of the modified polymerases of claim 47.

Claim 66 (previously presented): The kit of claim 65, further comprising one or more dideoxyribonucleoside triphosphates.

Claim 67 (previously presented): The kit of claim 65, further comprising one or more deoxyribonucleoside triphosphates.

Claim 68 (previously presented): The kit of claim 66, further comprising one or more deoxyribonucleoside triphosphates.

Claim 69 (previously presented): The polymerase of claim 14, wherein Arg722 is substituted with an amino acid selected from the group consisting of Lys, His, Asn, Tyr, and Leu.

Claim 70 (cancelled)

Claim 71 (currently amended): The polymerase of claim 1 or claim 2 [[70]], wherein Arg722 is substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, and Phe730 is substituted with an amino acid selected from the group consisting

of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr and Val.

Claim 72 (previously presented): The polymerase of claim 71, wherein Arg⁷²² is substituted with an amino acid selected from the group consisting of Lys, Gln, His, Asn, Tyr, and Leu.

Claim 73 (previously presented): The polymerase of claim 71, wherein Phe⁷³⁰ is substituted with Tyr.

Claim 74 (previously presented): The polymerase of claim 71, wherein Arg⁷²² is substituted with an amino acid selected from the group consisting of Lys, Gln, His, Asn, Tyr, and Leu, and Phe⁷³⁰ is substituted with Tyr.

Claim 75 (previously presented): The polymerase of claim 17, wherein Lys⁷²⁶ is substituted with Arg.

Claim 76 (previously presented): The polymerase of claim 57, wherein Arg⁷²² is substituted with an amino acid selected from the group consisting of Lys, His, Asn, Tyr, and Leu.

Claim 77 (cancelled)

Claim 78 (currently amended): The polymerase of claim 47 [[76]], wherein Arg722 is substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, and Phe730 is substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr and Val.

Claim 79 (currently amended): The polymerase of claim 78 [[76]], wherein Arg722 is substituted with an amino acid selected from the group consisting of Lys, Gln, His, Asn, Tyr, and Leu.

Claim 80 (currently amended): The polymerase of claim 78, wherein Phe730 is substituted with Tyr [[and]].

Claim 81 (previously presented): The polymerase of claim 78, wherein Arg722 is substituted with an amino acid selected from the group consisting of Lys, Gln, His, Asn, Tyr, and Leu, and Phe730 is substituted with Tyr.

Claim 82 (previously presented): The polymerase of claim 58, wherein Lys726 is substituted with Arg.